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HEPARIN CAUSES PLATELET DYSFUNCTION AND INDUCES FIBRINOLYSIS BEFORE THE INSTITUTION OF CARDIOPULMONARY BYPASS

BY

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ABSTRACT

Background: Platelet dysfunction and increased fibrinolysis are the most important etiologic factors in the hemostatic defect observed following the institution of cardiopulmonary bypass. This study examined the effects of heparin *per se*, administered prior to the institution of cardiopulmonary bypass, on platelet function and fibrinolysis.

Methods: Sampling was performed in 55 patients undergoing cardiac surgery before and 5 minutes after the routine administration of heparin, *prior* to the institution of cardiopulmonary bypass.

Results: Heparin administration resulted in a significant prolongation of the bleeding time (from 6.3±2.1 to 12.6±4.9 minutes, $p<0.0001$), a significant reduction in the level of shed blood thromboxane B_2 (from 1152±669 to 538±187 pg/0.1 ml, $p=0.00002$), an increase in the plasma levels of plasmin (from 50.2±1.0 to 99.3±39.9 $p=0.00001$) and D-dimer (from 571.3±297.1 to 698.5±358.6, $p=0.05$). There were no significant differences before and after heparin administration in the plasma levels of fibrinogen, plasminogen, tissue plasminogen activator, antiplasmin, antithrombin III, and von Willebrand factor.

Conclusion: Heparin, independent of cardiopulmonary bypass, causes both platelet dysfunction and increased fibrinolysis. The use of an alternative anticoagulant or a lower dose of heparin in conjunction with heparin-coated surfaces might improve the hemostatic balance during open heart surgery.

INTRODUCTION:

The institution of cardiopulmonary bypass alters hemostasis and results in increased postoperative bleeding (1). Platelet dysfunction and increased fibrinolysis are two mechanisms that are primarily responsible for the hemostatic dysfunction induced by cardiopulmonary bypass (1). Although contact with the extracorporeal circuit results in platelet loss secondary to platelet activation, secretion, and degranulation, the resultant thrombocytopenia encountered in the majority of patients undergoing cardiopulmonary bypass is not severe enough to account for the platelet dysfunction observed in these patients, which is manifested by a marked prolongation of the postoperative bleeding time (2). It was postulated that cardiopulmonary bypass induced platelet dysfunction by altering the platelet membrane receptors GP 1b and GP IIb/IIIa. A recent collaborative study from our institution demonstrated that the membrane receptors were intact in the platelet circulating during cardiopulmonary bypass (3). It suggested that factors extrinsic to the platelet might be important determinants of the platelet dysfunction observed during and following cardiopulmonary bypass. Hypothermia, for example, has been clearly shown to prolong the bleeding time and to reduce the platelet production of thromboxane A2 in patients undergoing cardiopulmonary bypass (4). Heparin, which is universally used in patients undergoing cardiopulmonary bypass, is another extrinsic factor which may influence platelet function in these patients. Heparin has also been shown to have profibrinolytic activity (5). Hence, heparin may contribute to the hemostatic abnormality of cardiopulmonary bypass not only by its inhibition of thrombin, but also by inducing platelet dysfunction and by promoting fibrinolysis. This investigation was carried out to elucidate the effect of the administration of heparin prior to the institution of cardiopulmonary bypass on platelet function and fibrinolytic activity in patients

undergoing cardiac surgery.

MATERIALS AND METHODS:

The patient population: This study was conducted in 55 patients (54 males,1 female) undergoing cardiac surgery at the West Roxbury Veterans Affairs Medical Center. The mean age (± Standard Deviation) of the patients was 64±8.7 years. The operations performed included isolated coronary artery bypass grafting (CABG, n=38), isolated valve replacement (n=5), and valve replacement with CABG (n=12). All patients signed consent forms approved by the institutional review board. No patient had a history suggestive of an underlying hemostatic disorder. Patients were not entered into the study if they had a history of aspirin, anti-inflammatory, or fibrinolytic drug intake within one week prior to the operation. All patients were premedicated with droperidol and fentanyl citrate (Innovar) and diphenhydramine hydrochloride (Benadryl). Anesthesia was induced with fentanyl and lidocaine and maintained with fentanyl and halothane. After an initial heparin dose of 4 mg/kg, cardiopulmonary bypass was begun. A membrane oxygenator and a circuit primed with lactated Ringer's solution were used. Subsequent heparin dosages were determined according to the activated clotting time, which was maintained greater than 600 seconds. After discontinuation of cardiopulmonary bypass, heparin was neutralized with protamine sulfate given in a ratio of 0.5 mg of protamine to 1.0 mg of the initial heparin dose and 1.0 mg of protamine to 1.0 mg for the subsequent heparin dosages. Heparin reversal was also monitored by the measurement of the activated clotting time.

Blood samples and assays: After opening the chest and prior to cannulation for cardiopulmonary bypass, two arterial blood samples were obtained, one before and one five minutes after the administration of heparin. Blood was collected for the measurement of

hematocrit and platelet count employing a Coulter ZBI Counter (Counter Electronics, Hialeah, Fla.). vWF antigen concentration was measured with enzyme-linked immunosorbent assay (6). vWF Multimer distribution was assessed by separating plasma proteins electrophoretically on a SDS=1% agarose gel using a continuous buffer (7). vWF antigen was identified by incubating the gel with ^{125}I-anti vWF antibody followed by autoradiography. Each sample was tested at least twice on separate gels to ensure reproducibility. Multimer distribution was assessed by visual inspection of the autoradiograph. Plasminogen and plasmin activity in plasma were measured employing the same substrate (2). Measurements of t-PA were made using enzyme-linked immunosorbent assay (ELISA). Samples for measurement of fibrinogen were collected in 3.8% sodium citrate tubes, centrifuged at 1200g for 10 minutes. After neutralization with protamine, fibrinogen was measured using a Coag-a-Mate X2 photo optical instrument (General Diagnostics). Heparin levels were measured with a chromogenic substrate. D-dimer, a breakdown product of fibrin, was measured by an enzyme-linked immunsorbent assay (ELISA) with a monoclonal antibody (2). Chromogenic assays were used for the measurement of antithrombin III and antiplasmin.

Measurement of the bleeding time and the shed blood thromboxane B_2: The bleeding time was measured from the lateral aspect of the volar surface of the forearm according to the method of Babson and Babson (8) with the Simplate II bleeding time device (General Diagnostics, Organon Teknika Corp., Durhan, N.C.). With every bleeding time measurement, local skin temperature was recorded by placing a surface skin thermistor (Skin Temperature sensor, Mon-A-Therm, Inc., St. Louis, MO.) within a few millimeters of the bleeding time site. Concomitant with bleeding time and temperature measurements, shed blood obtained from the bleeding time site

every 30 seconds was aspirated through a blunt needle into a tuberculin syringe coated with heparin (1000 U/ml) and containing 20 µl of ibuprofen for 1 ml of blood (1.9 mg/ml). Each template produced two skin incisions, and two templates were used at each period, one for measurement of the duplicate bleeding times and the recording of the mean value, and the other for collection of the shed blood from the skin incision. A volume of 0.6 ml of blood was collected from the bleeding time site for these measurements. The blood samples were kept on ice until they were centrifuged at 1650g (3000 rpm) in a Sorvall GLC-3 centrifuge (DuPont Company, Wilmington, DE.) for 10 minutes. The plasma was removed and frozen at -80°C until assays for thromboxane B_2 levels were done. Assays were performed with thromboxane B_2 (iodine 125) radioimmunoassay kits (New England Nuclear Corp., Boston, MA)

Data analysis: The bleeding time was corrected for temperature applying the factor described by Valeri et al (9). The plasma proteins were corrected for hemodilution as described previously (1). Comparisons were made between data obtained before and after the administration of heparin using the paired student t test. Statistical significance was set at $P \leq 0.05$. Correlations were performed employing linear regression analysis. Analyses were done using SAS Statistical Software (SAS Institute Inc., Cary, NC). The mean values in the narrative and the tables are shown ± their standard deviation; in the figures, they are shown ± their standard error.

RESULTS

Skin temperature, hematocrit and platelet count: The skin temperature at the site of the bleeding time determination drifted downward by an average of 1°C (Figure 1). This drift was ascribed to progressive cooling of the skin secondary to a low ambient temperature in the operating room and to the opening of the chest. In the 55 patients it averaged 29.7±1.3°C before

heparin and 28.7+1.7 C after heparin. This difference, though small, was highly significant ($p=0.0004$). The hematocrit before the administration of heparin was 34.31+5.04%. Post heparin it was 32.52+4.89% ($p=0.163$). The platelet count prior to heparin administration in 55 patients ranged from 64,000 to 399,000/ul. It averaged 212,833+66,569/ul. Five minutes after heparin administration it averaged 198,040+56,451/ul ($p=0.24$).

Heparin concentration and ACT: The activated clotting time (ACT) prior to heparin administration ranged from 105 to 201 and averaged 137.9+19.2 seconds. In most of the patients, heparin was administered to achieve an ACT of more than 600 seconds. As a result, the ACT after the administration of the first dose of heparin ranged from 479 to 1000 seconds and averaged 870+168 seconds. Heparin concentration in the blood prior to heparinization was 0.22+0.11 IU/ml. After the first dose, it ranged from 0.2 to 9.4 IU/ml and averaged 6.26+1.53 IU/ml. There was no correlation between the ACT and the blood heparin concentration ($r=0.09$) following the administration of the first dose of heparin.

Platelet Function: Platelet function parameters before and 5 minutes after heparin administration are shown in Table 1. The bleeding time prior to heparin administration ranged from 3.5 to 10.5 minutes and averaged 6.3 minutes. Following the administration of heparin, the bleeding time increased in all but two patients (Figure 2); it averaged 12.6 minutes. This was a marked and significant increase compared to the pre-heparin level ($p<0.00001$). The administration of heparin also resulted in a marked fall in the level of thromboxane B2 in the blood shed from the site of the bleeding time determination. Paired data were obtained in 32 patients; their results are shown in Table 1 and Figure 2. The mean shed blood thromboxane B2 prior to heparin was 11.5 ng/ml. Post heparin, it fell to 5.4 ng/ml ($p=0.00002$). There was a moderate but significant correlation between

the bleeding time and the shed blood thromboxane B2 ($r=0.43$, $p<0.0005$). There were no significant negative correlations between the skin temperature and the changes in the corrected bleeding time and the shed blood thromboxane B2 level. Therefore, the changes in bleeding time and thromboxane B2 were not due to the slight fall in skin temperature that was observed between the pre- and post-heparin time points.

von Willebrand Factor antigen concentration was measured before and 5 minutes after heparin administration in 15 patients. As shown in Table 1, the vWF antigen concentration before and after heparin were not significantly different. The vWF multimer distribution was not altered in patients after infusion of heparin, as measured by visual inspection of vWF antigen on autoradiographs (Figure 3).

Fibrinolytic Activity: Table 2 displays the results of fibrinolytic and related parameters before and after the administration of heparin. Plasmin was measured in 16 patients. As shown in Figure 4, heparin administration resulted in a ten-fold increase in plasmin from a pre-heparin average of 11.8 U/L to a post-heparin average of 125.4 U/L ($p=0.0001$). In these patients, the pre- and post-heparin administration levels of plasmin correlated significantly with the pre- and post-heparin bleeding times ($r=0.75$, $p<0.005$) (Figure 5). In 51 patients in whom D-dimer measurements were made, the administration of heparin elicited an increase in D-dimer in 39 (76%) and no change or a decrease in 12 (23%) (Figure 4). On the average, there was a modest but significant increase in the D-dimer from a pre-heparin mean of 571.3 ug/ml to a post-heparin mean of 698.5 ug/ml ($p=0.05$). As shown in Table 2, the administration of heparin elicited no significant changes in the plasma levels of fibrinogen, tissue plasminogen activator, plasminogen, antiplasmin, and antithrombin III.

This study demonstrates that, in patients undergoing open heart surgery, the administration of heparin, prior to the institution of cardiopulmonary bypass, causes platelet dysfunction (manifested by a prolongation of the bleeding time and a reduction in the ability of the platelet to produce thromboxane A2 in vivo) and increased fibrinolysis (manifested by increased plasma levels of plasmin and D-dimer). Hence, heparin contributes to the hemostatic defect observed in patients undergoing cardiac surgery not only by inhibiting coagulation through its effect on antithrombin III, but also by eliciting direct adverse effects on the platelet and the fibrinolytic system.

Platelet dysfunction in open heart surgery and the effect of heparin

It has been long recognized that patients undergoing cardiac surgery have increased postoperative bleeding. This has been attributed to a cardiopulmonary bypass-induced hemostatic defect which was thought to be due mostly to platelet dysfunction and, to a lesser extent, increased fibrinolysis (1,10). Since the magnitude of thrombocytopenia encountered in the course of cardiac surgery is not severe enough to account for the marked extension of the bleeding time observed during and following cardiopulmonary bypass (2), investigations in this field have focused on specific platelet abnormalities that might be induced by cardiopulmonary bypass. The platelet defect of cardiopulmonary bypass has been postulated to be due to a loss of the platelet membrane receptors responsible for platelet adhesion and aggregation. Loss of platelet membrane receptors for both the vWF and fibrinogen were reported during and following cardiopulmonary bypass (11-13). However, the methods with which the platelets were prepared in these studies involved centrifugation and gel filtration of the platelets prior to assay, thereby introducing the possibility of an artifactual *in vitro* decrease in platelet surface GPIb-IX complex (the vWF

receptor) and GPIIb-IIIa complex (the fibrinogen receptor) as a result of proteolysis or activation. In a study of 20 patients undergoing cardiac surgery, we utilized a flow cytometric method that allowed us to study the platelet GPIb-IX and GPIIb/IIIa in whole blood, thereby avoiding potential artefactual reductions in platelet surface glycoprotein receptors. With this method, we demonstrated that cardiopulmonary bypass did not result in a decrease in the platelet surface expression of neither the GPIb-IX complex nor the GPIIb-IIIa complex (3). This study underscored the possibility of factors *extrinsic* to the platelet itself that might contribute to the platelet dysfunction observed in patients undergoing cardiac surgery. Two such factors are hypothermia and heparin. In a study of 37 patients undergoing cardiopulmonary bypass, we confirmed the adverse effect of hypothermia on platelet function by demonstrating that hypothermia resulted in a significant prolongation of the bleeding time and a significant reduction in the ability of the platelet to produce thromboxane A2 (4).

Platelet function in the present study was assessed by the measurement of the bleeding time and the level of thromboxane B_2 in the blood shed from the skin at the site of the bleeding time determination. Despite its limitations, the bleeding time continues to be a reliable indicator of platelet function and platelet-vessel wall interaction (1). The measurement of the shed blood thromboxane B_2 has also been shown to be a reliable indicator of platelet function (2,14). It has the distinct advantage of reflecting *in-vivo* platelet function, making it an ideal measurement for use in patients undergoing cardiopulmonary bypass (2,3,4). Both measurements indicated a marked reduction in platelet function following the administration of heparin. Although it has been long recognized that heparin caused a prolongation of the bleeding time (15), its recognition as an etiologic factor in postbypass hemorrhage is only recent (1,16).

The mechanism with which heparin might cause platelet dysfunction was partially addressed in our previous study in which whole blood flow cytometry was employed in the characterization of the platelet membrane glycoproteins Ib and IIb-IIIa and platelet activation before and following cardiopulmonary bypass (3). That study provided evidence that heparin suppressed platelet activation *in vivo* via inhibition of *endogenous* thrombin, the latter being the most important platelet agonist *in vivo*.

Another possible mechanism for the inhibition of platelet function by heparin is through an inhibition of von Willebrand factor activity. Ristocetin is a cationic antibiotic which induces in vitro binding of vWF to its receptor on platelet GP1b. Sobel et al demonstrated that the intravenous administration of heparin to patients before open heart surgery reduced ristocetin cofactor activity by 58% (17). This impairment of vWF-dependent platelet function was closely related to plasma heparin levels but not to plasma vWF levels. It appeared to be caused by direct binding of heparin to vWF in solution, but may also be a consequence of heparin binding directly to the platelet surface (18). In the present study, we have demonstrated that the concentration and multimer distribution of the vWF were unaffected by the administration of heparin. Hence our data is consistent with the hypothesis that heparin reduces platelet function by preventing the vWF from binding to its receptor on the GPIb on the platelet.

The third mechanism by which heparin can induce platelet dysfunction is through its effect on plasmin. This is explained in the section below.

Fibrinolysis during cardiopulmonary bypass and the effect of heparin

There are a number of pathways that could lead to increased fibrinolysis during cardiopulmonary bypass surgery, including the activation of kallikrein and the release of t-PA

from the endothelial cell (1). Fibrin(ogen) degradation products (FDP) and D-dimer increase during and following cardiopulmonary bypass (1,19). Administration of aprotinin during cardiopulmonary bypass prevents the formation of FDP and the reduction in α_2-antiplasmin activity (20). It also reduces the bleeding time (21). These observations, the mechanism of action of aprotinin (22), and the clinical effectiveness of aprotinin in decreasing blood loss (21) strongly suggest that fibrinolysis is important in the hemorrhagic diathesis associated with cardiopulmonary bypass surgery. The present study is the first to demonstrate that, in patients *prior to the institution of cardiopulmonary bypass*, the administration of heparin increases fibrinolysis as evidenced by a uniform increases in plasmin and a partial but significant increase in D-dimer. Heparin and its fractions, however, have been known to possess profibrinolytic activities for over a decade (5). Fareed et al reported an increase in tissue plasminogen activator levels in human volunteers after the institution of intravenous and subcutaneous heparin over a period of 10 days (daily dose of 7500 units). They postulated a number of profibrinolytic actions of heparin and its fractions (5). Several recent studies have shown that heparin and heparin-like compounds stimulated cell surface plasminogen activation by 10- to 17-fold (23). Since this interaction occurred at the cell surface level, it would be unlikely that the profibrinolytic effect of heparin would be reflected by the levels of plasminogen in the plasma. Hence, in our study, we did not show any change in the plasma levels of plasminogen with the administration of heparin. The lack of a significant increase in t-PA following the administration of heparin did not denote a lack of profibrinolytic activity because the *plasma* level tPA antigen may not correlate with the functional activity of the molecule in patients undergoing cardiopulmonary bypass. The rise in plasmin and D-dimer following the administration of heparin indicated an increase in profibrinolytic activity.

It is of note that plasmin, fibrin degradation products, and D-dimer have all been shown to interfere with platelet aggregation, presumably through a proteolytic effect on the platelet GPIIIa in the case of plasmin (24) and by competing with fibrinogen for GPIIb/IIIa binding in the case of the degradation products. Hence the generation of plasmin, stimulated by heparin, provides another possible mechanism by which heparin impairs platelet function as well. A confirmatory finding of this mechanism is the significant correlation which was observed in this study between the bleeding time and the plasma levels of plasmin.

Clinical implications

The hemostatic defect observed in patients undergoing cardiac surgery is not fully reversed with the administration of protamine and the discontinuation of cardiopulmonary bypass. In fact, D-dimer levels increase after the administration of protamine, and the bleeding time continues to be significantly elevated in the hours following the discontinuation of cardiopulmonary bypass (1,2). The bleeding time at two hours following cardiopulmonary bypass was shown to be an important determinant of postoperative blood loss (2). Interventions aimed at reducing postoperative platelet dysfunction and fibrinolysis are likely to reduce postoperative blood loss in patients undergoing cardiac surgery. Because a variety of complex factors can potentially contribute to the hemostatic defect observed in the initial postoperative hours in these patients, it is very difficult to appreciate the specific contribution of the administration of heparin per se to this defect. Nevertheless, the present study raises questions as to whether an alternative anticoagulant or a lower dose of heparin might improve the hemostatic balance during open heart surgery. In the present study, the ACT was kept on the high side to achieve full suppression of thrombin. Significantly lower ACT levels have been employed in conjunction with heparin-coated

surfaces. It remains to be determined whether combining low-dose heparin with heparin-coated surfaces would suppress thrombin adequately and, at the same time, reduce the adverse effect of heparin on platelet function and fibrinolysis. It also remains to be determined whether an alternative anticoagulant that would not interfere with either the platelet function or the fibrinolytic process can improve post-operative hemostasis in the cardiac surgical patient.

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TABLE 1: PLATELET FUNCTION PARAMETERS BEFORE AND 5 MINUTES AFTER THE ADMINISTRATION OF HEPARIN

	n	Before Heparin	After Heparin	p
Bleeding Time (minutes)	54	6.3 ± 2.1	12.6 ± 4.9	0.00001
Shed Blood TxB_2 (pg/0.1ml)	32	1152 ± 669	538 ± 187	0.00002
vWF antigen (%)	15	172 ± 48	136 ± 55	0.64

TABLE 2: PARAMETERS RELATED TO FIBRINOLYSIS BEFORE AND 5 MINUTES AFTER THE ADMINISTRATION OF HEPARIN.

	n	Before Heparin	After Heparin	p
Plasmin (U/L)	16	11.8+9.7	125.4+34.8[b]	0.0001
D-dimer (μg/ml)	51	571.3 ± 297.1	698.5 ± 358.6*	0.05
t-PA (ng/ml)	25	7.12 ± 5.1	6.47 ± 5.17*	NS
Fibrinogen (mg/dl) [b]	28	387.7 ± 84.1	372.8 ± 109.1*	NS
Plasminogen (%)	19	79.3 ± 12.8	84 ± 14*	NS
Antiplasmin (%)	19	75.2 ± 8.4	71 ± 21*	NS
Antithrombin III (%)	28	80 ± 12	80 ± 12*	NS

* value corrected for dilution, ** samples neutralized with protamine

LEGENDS TO FIGURES

Figure 1. The arm skin temperature at the site of the bleeding time determination before and 5 minutes after the administration of heparin in 55 study patients. The mean + SEM for each time point are shown.

Figure 2. The bleeding time and the shed blood thromboxane B2 in the patients in whom paired measurements were obtained before and 5 minutes after the administration of heparin. The mean + SEM for each time point are shown.

Figure 3. Autoradiograph of vWF in normal pool plasma (N), and from three representative patients out of 15, before (A) and after (B) infusion of heparin. Note no change in the multimer distribution between the two time points in all three patients.

Figure 4. The plasma levels of plasmin and D-dimer in the patients in whom paired measurements were obtained before and 5 minutes after the administration of heparin. The mean + SEM for each time point are shown.

Figure 5. Plasmin levels and bleeding times in patients pre and post heparin administration.

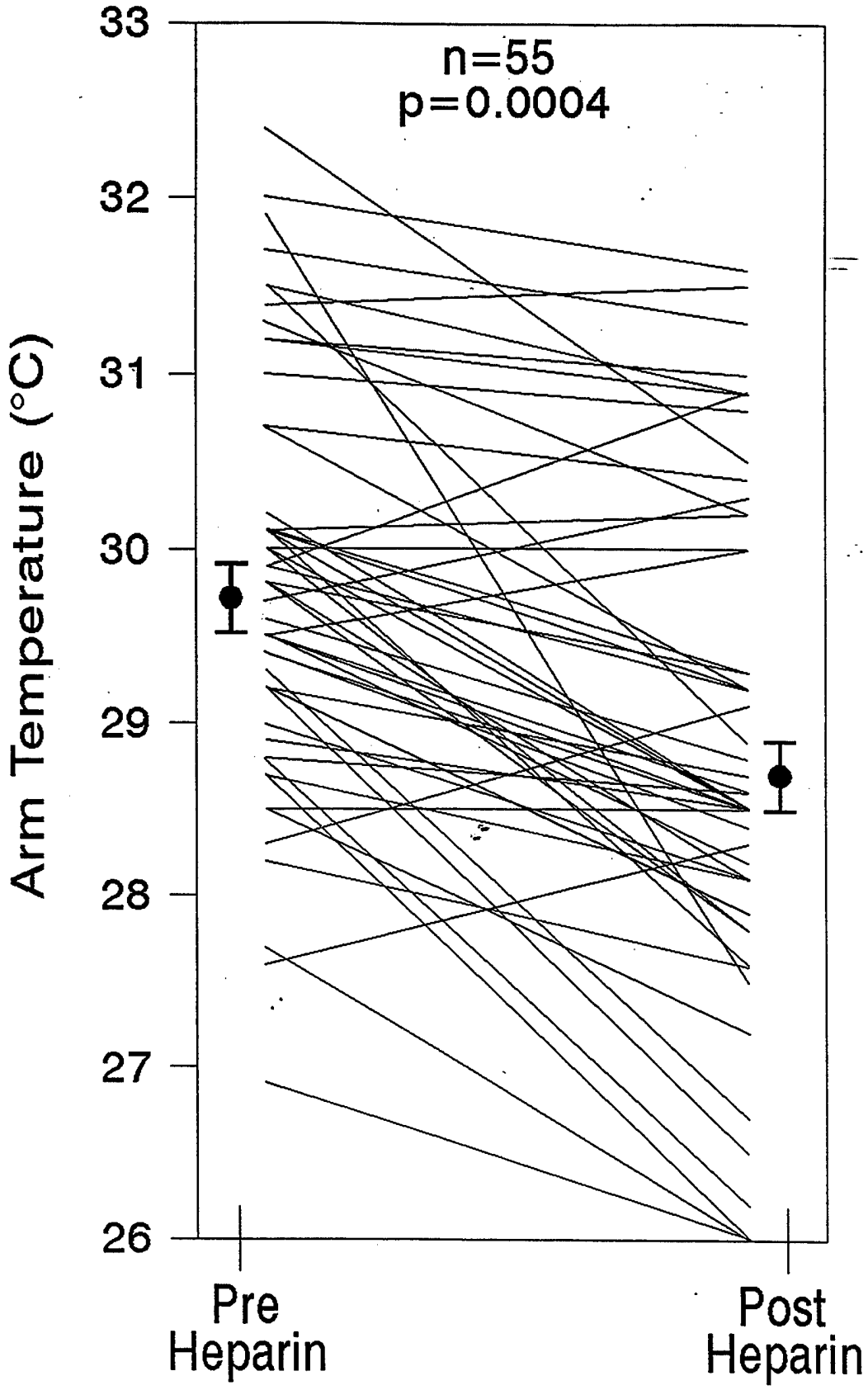
n=55
p=0.0004
33
32
31
30
29
28
27
26
Arm Temperature (°C)
Pre Heparin
Post Heparin

FIGURE 1

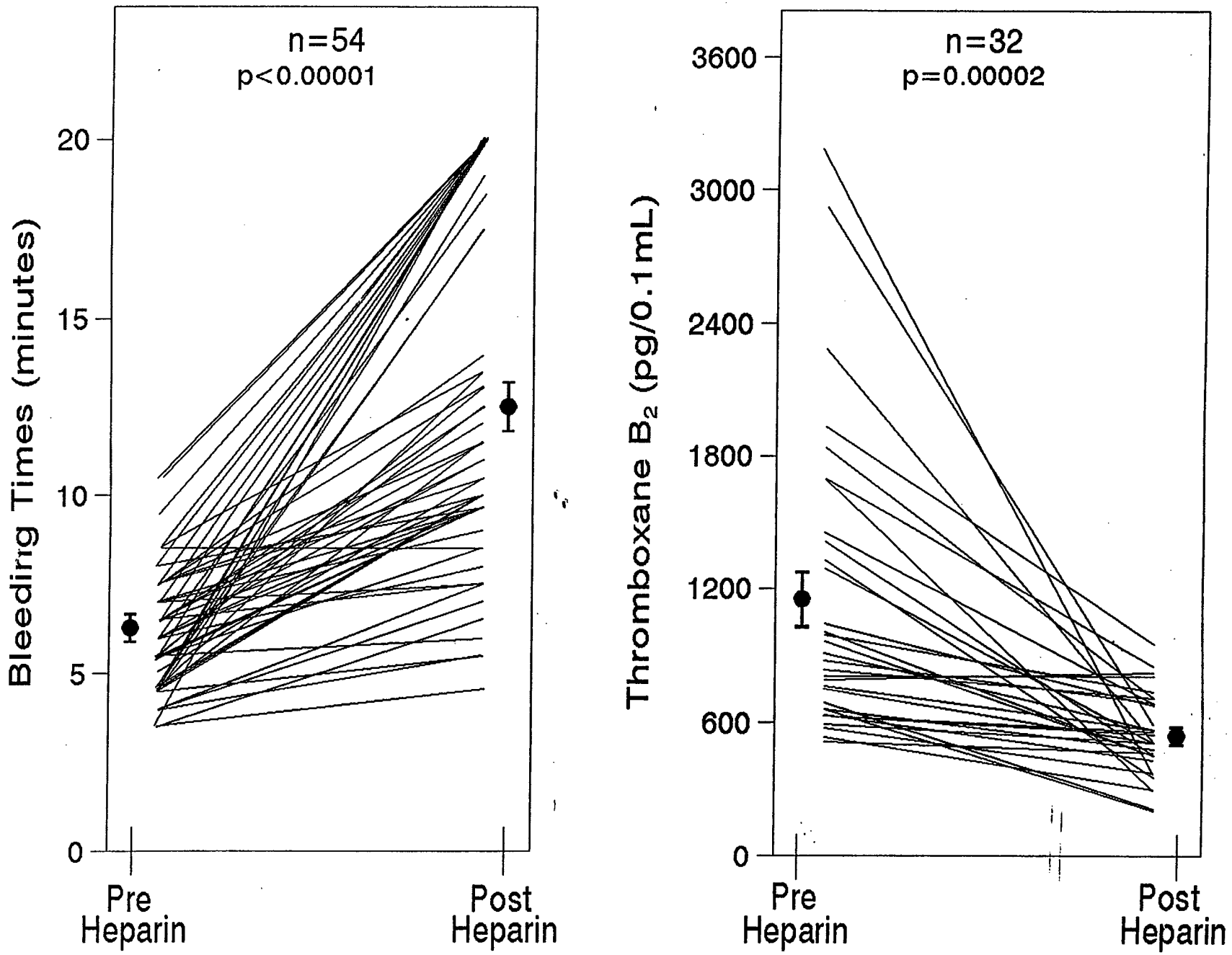
n=54
p<0.00001
Bleeding Times (minutes)
0
5
10
15
20
Pre Heparin
Post Heparin
n=32
p=0.00002
Thromboxane B_2 (pg/0.1mL)
0
600
1200
1800
2400
3000
3600
Pre Heparin
Post Heparin

FIGURE 2

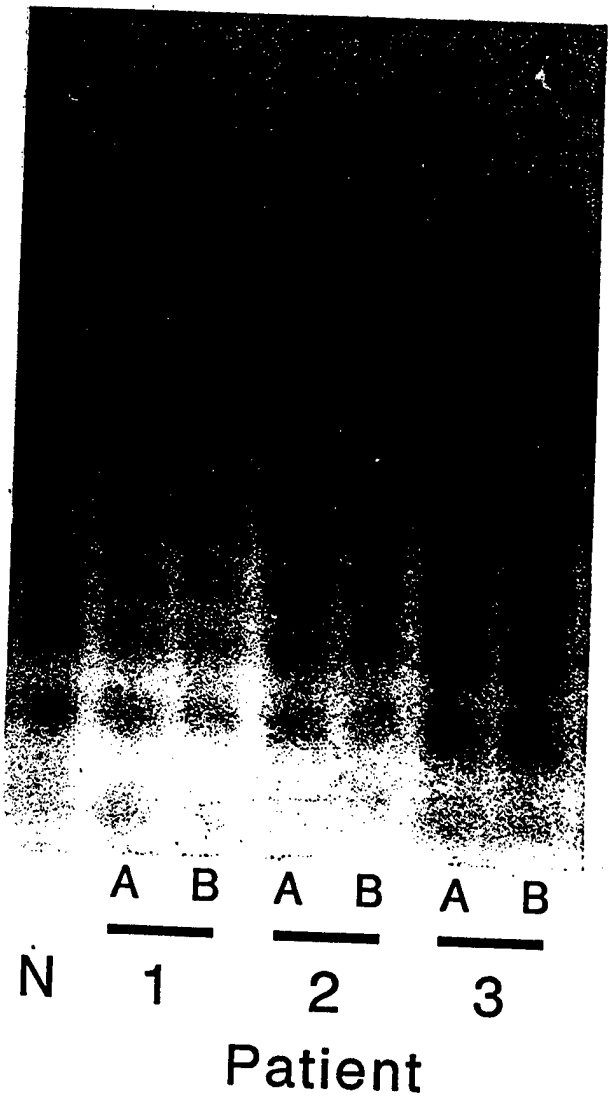

FIGURE 3

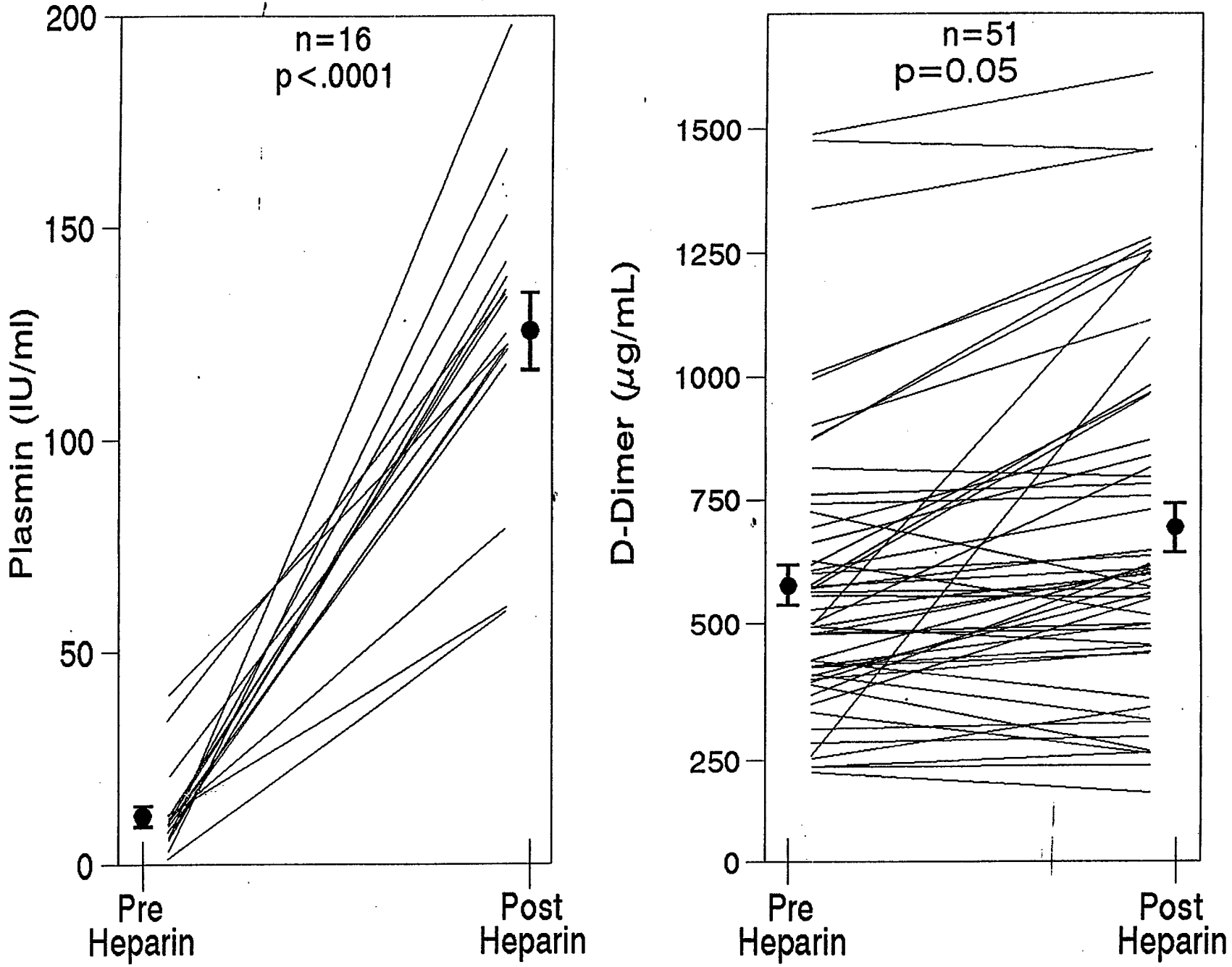
n=16
p<.0001
Plasmin (IU/ml)
200
150
100
50
0
Pre
Heparin
Post
Heparin
n=51
p=0.05
D-Dimer (μg/mL)
1500
1250
1000
750
500
250
0
Pre
Heparin
Post
Heparin

FIGURE 4

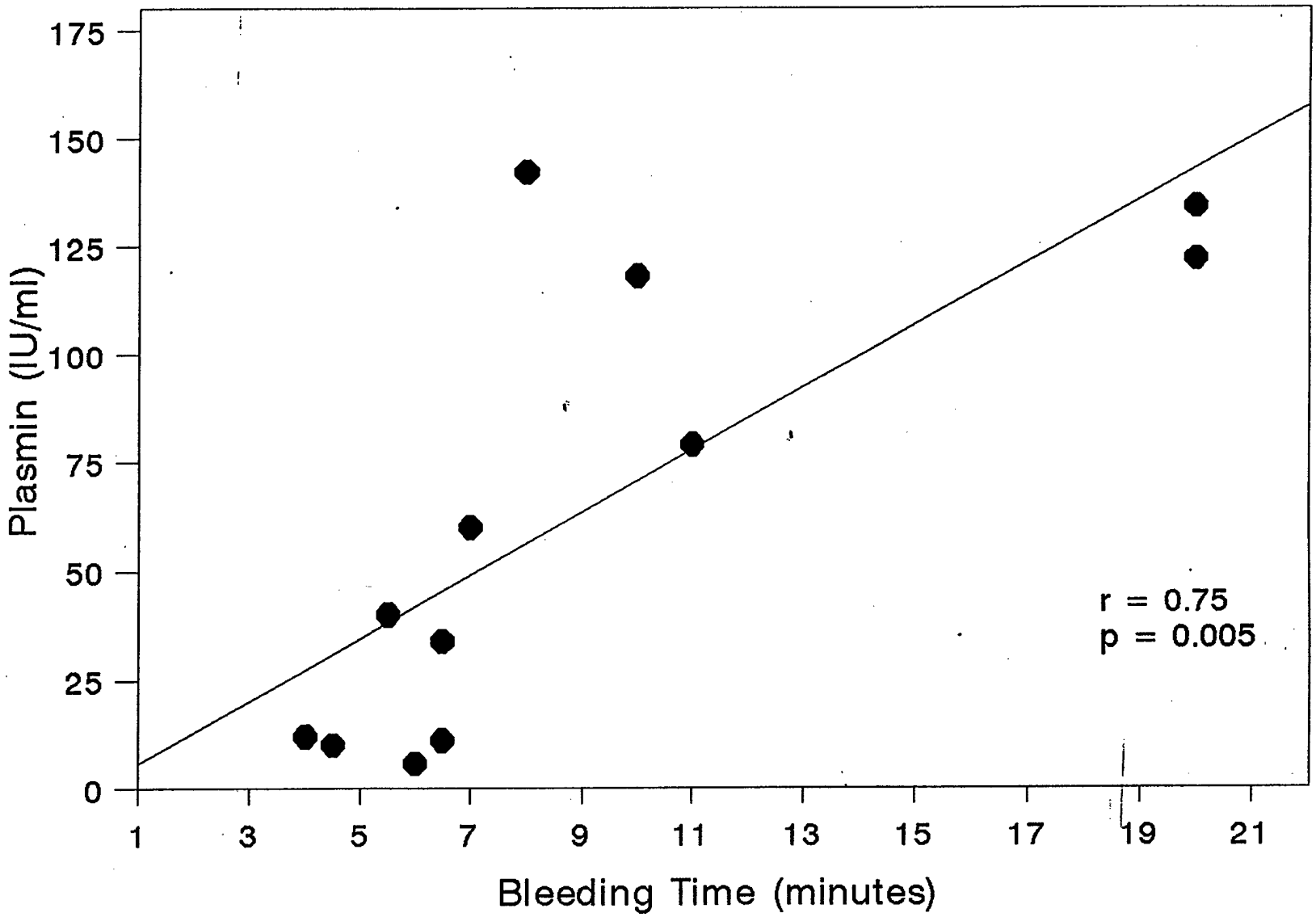
175
150
125
100
75
50
25
0
Plasmin (IU/ml)
1
3
5
7
9
11
13
15
17
19
21
Bleeding Time (minutes)
r = 0.75
p = 0.005

FIGURE 5